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Purification of Calmodulin from *Chlamydomonas*: Calmodulin Occurs in Cell Bodies and Flagella

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ABSTRACT Calmodulin has been purified from cell bodies of the green alga *Chlamydomonas* by Ca^{++}-dependent affinity chromatography on fluphenazine-Sepharose 4B. Calmodulin from this primitive organism closely resembles that from bovine brain in a number of properties, including (*a*) binding to fluphenazine in a Ca^{++}-dependent, reversible manner, (*b*) functioning as a heat-stable, Ca^{++}-dependent activator of cyclic nucleotide phosphodiesterase, and (*c*) electrophoretic mobility in SDS-polyacrylamide gels in both the presence and absence of Ca^{++}, which causes a shift in the relative mobility of calmodulin. Calmodulin has also been identified by the criteria of phosphodiesterase activation and electrophoretic mobility in both the detergent soluble "membrane plus matrix" and the axoneme fractions of *Chlamydomonas* flagella. Calmodulin is not associated with the partially purified 12S or 18S dynein ATPases of *Chlamydomonas*. The presence of calmodulin in the flagellum suggests that it is involved in one or more of the Ca^{++}-dependent activities of this organelle.

Calmodulin, or Ca^{++}-dependent regulator (CDR), is a small, heat-stable, Ca^{++}-binding protein, which modulates a large number of Ca^{++}-dependent events in eukaryotes (see references 13 and 46 for reviews). Calmodulin is an activator of several enzymes, including Ca^{++}-dependent cyclic nucleotide phosphodiesterase (12), Ca^{++}-activated adenylate cyclase (7), NAD kinase (2), myosin light-chain kinase (14, 47), and the Ca^{++}-stimulated ATPase of the erythrocyte plasma membrane (20, 27). In addition, calmodulin has been reported to be involved in the control of Ca^{++}-dependent microtubule assembly-disassembly (32), and has been localized in the spindle apparatus (1, 44), in microfilament bundles (16), and in microvillae (23).

Calcium ions are required for a number of processes which involve cilia and flagella. For example, in the green alga *Chlamydomonas*, Ca^{++} has a role in phototaxis (37), in the control of flagellar waveform (3, 24, 35), in the maintenance of flagellar length (29, 34), and in flagellar surface motility—the movement of particles associated with the flagellar membrane (4). In other organisms, Ca^{++} has a role in avoidance reactions (17), in chemotaxis (6), and in ciliary arrest (40) and flagellar quiescence (19). Recently, the presence of calmodulin has been reported in the cilia and axonemes of the ciliates *Tetrahymena* (26) and *Paramecium* (31). Calmodulin may, therefore, be involved in one or more of the Ca^{++}-dependent processes associated with cilia and flagella.

As a first step toward investigating the possible involvement of calmodulin in these processes, we have isolated a calmodulinlike protein from *Chlamydomonas* and determined its distribution in various flagellar fractions. The protein appears to be a true calmodulin on the basis of several biological and biochemical properties. It occurs in the cell body, in the detergent-soluble membrane plus matrix fraction of the flagellum, and in the flagellar axoneme.

MATERIALS AND METHODS

Isolation of Cell and Flagellar Fractions

Chlamydomonas reinhardii (strain 1132D) was grown in synchronous cultures of up to 96 liters, and the cells harvested, washed, and deflagellated by the dibucaine procedure, all as previously described (45). Subsequent operations were carried out at 4°C unless stated otherwise. The cell bodies were separated from the detached flagella by centrifugation at 1,700 *g* (IEC 253 head, 2,600 rpm) for 5 min. The pellet of cell bodies was used as the starting material for purification of calmodulin (see below). The supernate, containing the flagella, was collected, underlayered with a cushion of 25% sucrose in 10 mM HEPES, 5 mM $MgSO_4$, and 1 mM DTT at pH 7.5, and recentrifuged for 10 min at 2,750 *g* (IEC 253 head, 3,300 rpm) to remove any remaining cell bodies. The cell-free supernate from this centrifugation was then spun at 31,000 *g* (Sorval SS-34 rotor, DuPont Instruments-Sorvall, DuPont Co., Newton, Conn., 16,000 rpm) for 20 min to pellet the flagella. For electrophoretic studies, the flagella were demembranated by resuspension in ice-cold 1% Nonidet P-40 (2 ml/ml packed cells) in HMDEK (30 mM HEPES, pH 7.5 at 23°C; 5 mM $MgSO_4$; 1 mM DTT; 0.5 mM Na_2EDTA; 25 mM KCl). After 10–20 min at room temperature, the suspension was recentrifuged as above; the resulting supernate, containing solubilized components of the flagellar membrane and matrix, and the pellet, containing demembranated axonemes, were prepared for SDS-gel electrophoresis. For studies on phosphodiesterase activation, the flagella were demembranated by treatment with 0.04% Nonidet in HMDEK and the suspension centrifuged as above. The

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supernate was used for the membrane and matrix fraction; the axonemes were washed twice by resuspension in 1% Nonidet in HMDEK and once by resuspension in HMDEK before final resuspension in HMDEK. In some experiments, EGTA was substituted for EDTA in all solutions.

12S and 18S dynein ATPases were partially purified as described by Fay and Witman (18 and footnote 1). The dyneins were extracted from isolated *Chlamydomonas* axonemes by treatment with a solution containing 0.5 M KCl, 30 mM Tris (pH 7.5), 5 mM $MgSO_4$, 1 mM DTT, 0.5 mM Na_2EDTA, and 0.1 mM ATP. The resulting extract was dialyzed overnight against 30 mM Tris (pH 7.5), 0.5 mM Na_2EDTA, 1 mM DTT, and 25 mM KCl (TEDK), and the dyneins then separated by zone sedimentation in 5–20% linear sucrose density gradients in TEDK.

Preparation of Fluphenazine-Sepharose for Affinity Chromatography

Fluphenazine was coupled to Sepharose 4B by the bisoxirane method of Sundberg and Porath (38) as described by Charbonneau and Cormier (11). Fluphenazine·2 HCl was the kind gift of Mr. S. J. Lucania of the Squibb Institute (Princeton, N.J.).

Purification of Calmodulin

Chlamydomonas calmodulin was purified by a modification of the method of Charbonneau and Cormier for purification of peanut calmodulin (11). All operations were carried out at 4°C. Packed *Chlamydomonas* cell bodies were resuspended in 2 vol of a solution containing 50 mM Tris (pH 8.0 at 23°C), 1 mM β-mercaptoethanol, and 1 mM Na_2EDTA. The cells were broken open by sonication with a Branson model S75 Sonifier (Branson Sonic Power Co., Danbury, Conn.), and the suspension then centrifuged for 30 min at 8,700 *g* (Sorval SS-34 rotor [DuPont Instruments-Sorvall, DuPont Co.] 8,500 rpm). The supernate was collected, mixed with 1.1 vol of absolute ethanol, and stirred for 10 min. The suspension was then recentrifuged as above, the supernate collected, mixed with three times the initial supernatant volume of absolute ethanol, and stirred for 10 min. The mixture was recentrifuged as above, and the resulting pellet resuspended in column wash buffer (10 mM HEPES, 1 mM $CaCl_2$, 0.5 M NaCl, pH 7.0 at 23°C).

Samples in column wash buffer were applied to a 3.75 ml (0.5 cm i.d. x 12 cm) column of fluphenazine-Sepharose equilibrated with the same buffer. The column was then washed with this buffer until the A_{280} of the eluant dropped to base line level. Bound calmodulin was then eluted by application of a buffer containing 10 mM Tris (pH 8.0 at 23°C) and 10 mM EGTA. 1-ml fractions were collected at a rate of 30 ml/h; fractions showing absorbance after application of the EGTA-containing buffer were pooled, dialysed in benzoylated dialysis tubing against 1 mM β-mercaptoethanol, and stored frozen at −80°C. For electrophoretic analysis of individual column fractions, small portions were removed from some fractions before the peak fractions were pooled.

Phosphodiesterase Assay for Calmodulin

The ability of *Chlamydomonas* calmodulin to activate calmodulin-dependent cyclic nucleotide phosphodiesterase was determined by the method of Wolff et al.[2] This assay measures the conversion of G-^{3}H-cGMP to [^{3}H]guanosine by the coupled activities of bovine brain phosphodiesterase and calf intestinal alkaline phosphatase. Before the assay, samples of *Chlamydomonas* protein were heated for 2 min at 100°C.

The bovine brain calmodulin used in these studies was very generously provided by Dr. Donald Wolff of the College of Medicine and Dentistry of New Jersey-Rutgers Medical School. Calmodulin-depleted cyclic nucleotide phosphodiesterase was prepared from fresh calf brains as described by Watterson et al. (41). Alkaline phosphatase (Sigma P-4502) and cGMP (Sigma G-6129) were obtained from the Sigma Chemical Co. (St. Louis, Mo.); [8-^{3}H]-cGMP was obtained from the Amersham Corp. (Arlington Heights, Ill.). The assays shown in Fig. 1 were carried out in the laboratory of Dr. Wolff, using materials kindly supplied by him.

[1] Fay, R. B., and G. B. Witman. Isolation and polypeptide composition of three dynein ATPases from *Chlamydamonas* flagella. Manuscript in preparation.

[2] Wolff, D. J., J. M. Ross, P. N. Thompson, M. A. Brostrom, and C. O. Brostrom. 1980. Histone phosphatase activity modulated by the Ca^{++}-dependent regulator protein. I. Partial purification from brain and kinetic properties in dephosphorylation of mixed histones. Manuscript submitted for publication.

Acrylamide Gel Electrophoresis

SDS-polyacrylamide gel electrophoresis was performed in 12.5% acrylamide slab gels according to the method of Laemmli and Favre (28), except that the running buffer contained 3 g Tris, 14.4 g glycine, and 1 g SDS per liter, and the sample buffer contained, in addition to the usual components, either 1 mM $CaCl_2$ or 1 mM EGTA. Electrophoresis was carried out for 4 h at a constant power of 2 W/gel. Gels were stained in 0.25% Coomassie Blue in methanol:glacial acetic acid:water (5:1:5), and destained in 20% methanol, 7.5% acetic acid.

The purified bovine brain calmodulin used in our electrophoretic analyses was kindly provided by Dr. Thomas Vanaman of the Duke University Medical Center.

Protein Determination

Protein in crude fractions was determined by the method of Bradford (5) using Bio-Rad Protein Assay Dye Reagent (Bio-Rad Laboratories, Richmond, Calif.) and bovine gamma globulin as a standard.

Amounts of purified *Chlamydomonas* calmodulin were determined by quantitative densitometry of Coomassie Blue–stained acrylamide gels using purified bovine brain calmodulin as a standard (41). Gels were scanned directly with a Joyce-Loebl microdensitometer (Joyce, Loebl and Co., Ltd., Gateshead-on-Tyne, England) and peak areas of the tracings measured with a planimeter.

RESULTS

Purification of Calmodulin from Chlamydomonas

A number of antipsychotic drugs bind to calmodulin with high affinity (see reference 43 for review); this binding, which is highly selective, dependent upon Ca^{++} at neutral pH, and readily reversible by addition of EGTA, has been used as the basis for purification of calmodulin from a number of sources by affinity chromatography (11, 25). To determine if calmodulin could be purified from *Chlamydomonas* by affinity chromatography, an extract prepared from *Chlamydomonas* cell bodies was applied to a fluphenazine-Sepharose 4B column in the presence of 1 mM $CaCl_2$. With continued washing of the column, most of the applied protein was eluted, and the absorbance of the eluant dropped to zero (Fig. 1). When buffer containing EGTA was then applied to the column, a small peak of protein was eluted. Fractions from this peak contained a single low molecular weight polypeptide that closely resembled bovine brain calmodulin in its electrophoretic mobility (Fig. 1, inset). This protein was not detected in the main (nonbound) protein peak or in fractions between the two peaks.

Based on quantitative densitometry of SDS-acrylamide gels, approximately 0.55 mg of calmodulin was obtained from ~80-ml packed cells.

Heat Stability and Phosphodiesterase Activation

Two distinguishing features of calmodulin are heat stability and the ability to activate cyclic nucleotide phosphodiesterase in a Ca^{++}-dependent manner (12). To determine if these were also properties of *Chlamydomonas* calmodulin, the activity of calmodulin-depleted bovine brain phosphodiesterase was assayed in the presence of various amounts of the *Chlamydomonas* protein, which had been incubated for 2 min at 100°C. Fig. 2 shows the activation curve for the heat-treated *Chlamydomonas* calmodulin; this curve was very similar to that obtained for bovine brain calmodulin. Both *Chlamydomonas* calmodulin and bovine brain calmodulin increased the activity of the phosphodiesterase 10- to 11-fold. No stimulation occurred in the absence of Ca^{++}.

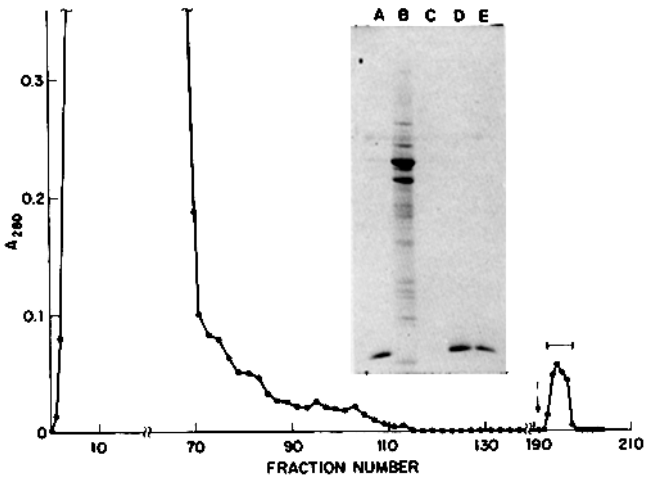

FIGURE 1 Chromatography of a *Chlamydomonas* extract on a fluphenazine-Sepharose column. 25 ml of extract containing 212 mg protein (obtained from ~50 ml packed cells) was applied to the column commencing with fraction 1. The column was then washed with 10 mM HEPES, 1 mM $CaCl_2$, 0.5 M NaCl, pH 7.0. Beginning with fraction 191 (arrow), the column was washed with 10 mM Tris, 10 mM EGTA, pH 8.0. 1-ml fractions were collected. Fractions 193–198 (bar) were pooled for further analysis. Inset: SDS-polyacrylamide gel of bovine brain calmodulin (*A*), and column fractions 50 (*B*), 180 (*C*), 194 (*D*), and 196 (*E*). 1 mM EGTA was added to each sample. Fractions 194 and 196 contained a single polypeptide, which resembled bovine brain calmodulin in its electrophoretic mobility. The faint bands in the top half of the gel are artifactual.

The specific activity of the purified *Chlamydomonas* calmodulin was ~33,000 U/mg.[3]

Effect of Ca^{++} on Electrophoretic Mobility

Recently, Burgess et al. (9) reported that the electrophoretic mobility of bovine brain calmodulin in SDS-polyacrylamide gels varied depending on whether electrophoresis was carried out in the presence or absence of Ca^{++}; this behavior apparently reflected the ability of calmodulin to bind Ca^{++} in the presence of SDS. To determine if Ca^{++} would also induce a change in the mobility of *Chlamydomonas* calmodulin in SDS-polyacrylamide gels, the protein was electrophoresed after addition of either 1 mM Ca^{++} or 1 mM EGTA to the sample buffer. As can be seen in Fig. 3, both bovine brain calmodulin and *Chlamydomonas* calmodulin migrated faster in the presence of Ca^{++} than in its absence. In the presence of 1 mM Ca^{++}, brain and *Chlamydomonas* calmodulins had apparent molecular weights of 18,000 and 17,500, respectively; in the absence of Ca^{++}, their respective apparent molecular weights were 19,400 and 19,600.

Presence of Calmodulin in Flagella

The fact that Ca^{++} changes the mobility of *Chlamydomonas* calmodulin in SDS-polyacrylamide gels can be used to identify tentatively this protein in crude preparations and cell fractions. Fig. 4 shows the results obtained when the axoneme and membrane plus matrix fractions of *Chlamydomonas* flagella were electrophoresed in the presence and absence of Ca^{++}. In the presence of Ca^{++}, a protein in the membrane plus matrix fraction very clearly comigrated with purified *Chlamydomonas* calmodulin at about 17,500 daltons; in the absence of Ca^{++}, this band completely disappeared and a new band appeared which coelectrophoresed with purified calmodulin at 19,600 daltons. Similarly, the flagellar axoneme contained a protein which comigrated with *Chlamydomonas* calmodulin under both conditions; however, calmodulin in this fraction was less obvious because of the presence of other proteins migrating in the same areas. A Ca^{++}-induced shift in mobility was not observed in any of the other proteins resolved in these fractions.

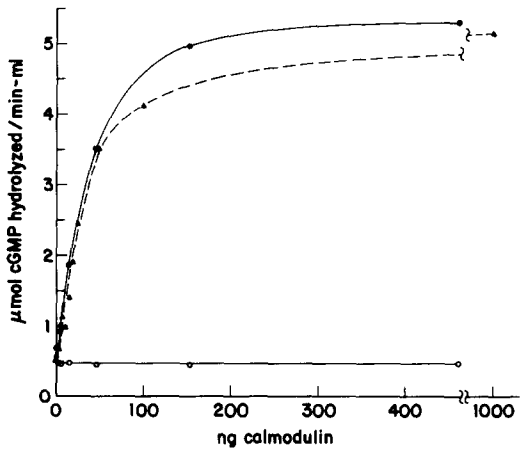

FIGURE 2 Activity of bovine brain cyclic nucleotide phosphodiesterase in the presence of various amounts of *Chlamydomonas* (●) or bovine brain (▲) calmodulin. All assays contained 0.042 mM Ca^{++}. Assays were also carried out with *Chlamydomonas* calmodulin in the presence of 0.042 mM Ca^{++} plus 1.6 mM EGTA (○). Phosphodiesterase activity is expressed as micromoles cGMP hydrolyzed per minute-milliliter of phosphodiesterase (12.5 mg protein).

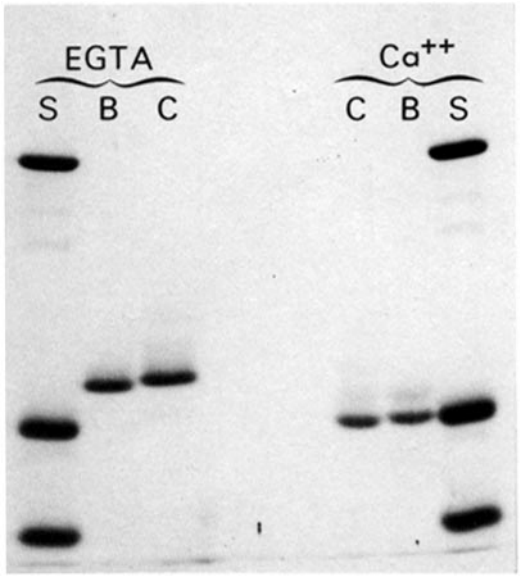

FIGURE 3 SDS-polyacrylamide gel electrophoresis of *Chlamydomonas* calmodulin (*C*), bovine brain calmodulin (*B*), and protein standards (*S*) after addition of 1 mM Ca^{++} or 1 mM EGTA to samples. Standards were cytochrome *c* (11,700), β-lactoglobulin (18,400), and carbonic anhydrase (29,000). The faint bands that migrated slightly behind both *Chlamydomonas* and bovine brain calmodulin appeared upon storage of the samples.

[3] One unit of calmodulin was defined as that amount which resulted in 50% maximum activation of a standard preparation of calmodulin-depleted phosphodiesterase.

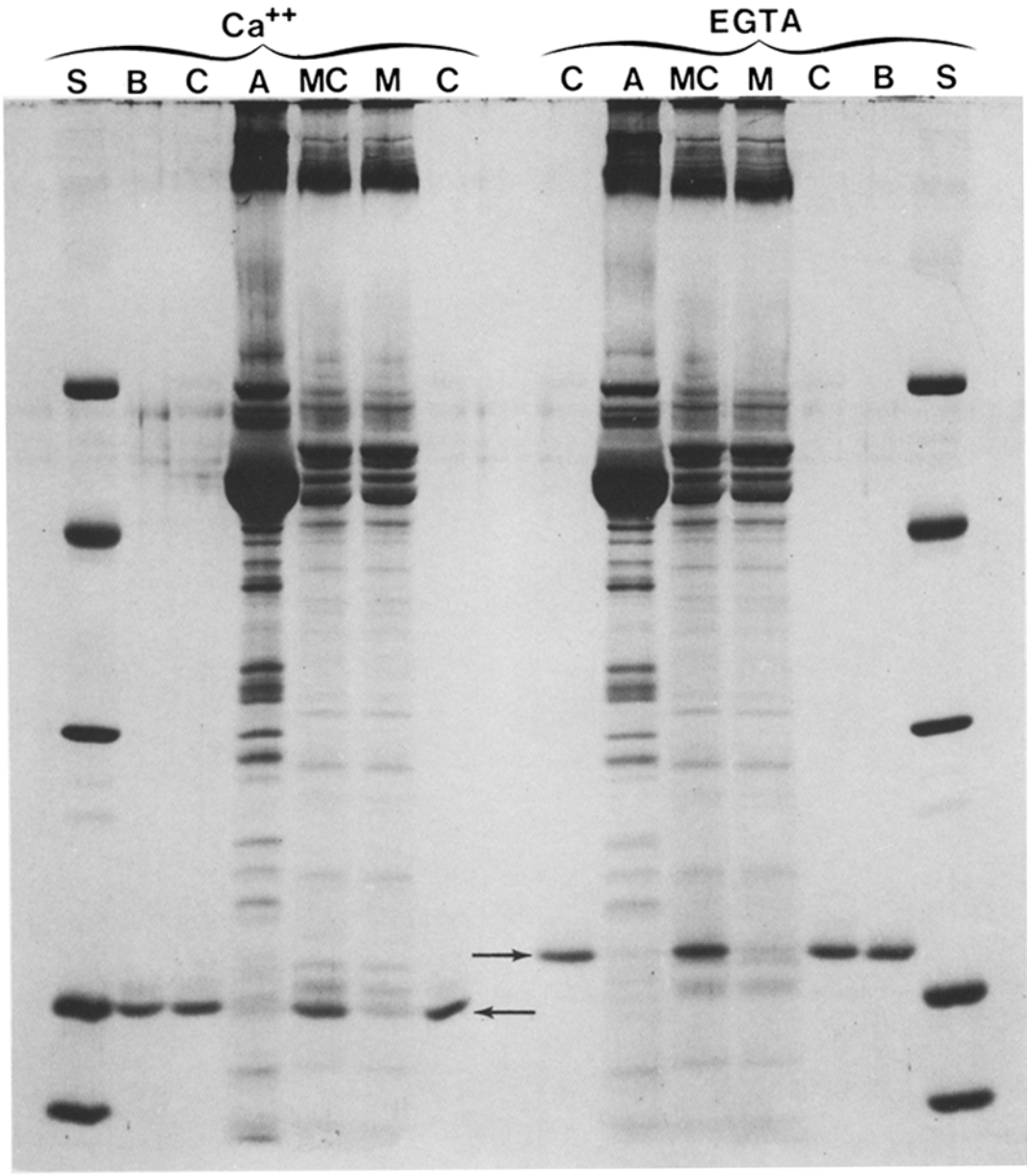

FIGURE 4 SDS-polyacrylamide gel electrophoresis of purified calmodulin and flagellar fractions in the presence and absence of Ca^{++}. *B*, 2 μg bovine brain calmodulin; *C*, 1.8 μg purified *Chlamydomonas* calmodulin; *A*, 40 μg isolated axonemes; *M*, 37 μg membrane plus matrix fraction; *MC*, 37 μg membrane plus matrix fraction and 1.4 μg of *Chlamydomonas* calmodulin; *S*, protein standards. Samples contained 1 mM Ca^{++} or 1 mM EGTA, as indicated. Arrows indicate positions of calmodulin under the two conditions. Protein standards were cytochrome *c* (11,700), β-lactoglobulin (18,400), carbonic anhydrase (29,000), ovalbumin (43,000), and bovine serum albumin (68,000).

Activation of Phosphodiesterase by Flagellar Fractions

To confirm that the flagellar fractions contained calmodulin, the isolated axoneme and membrane plus matrix fractions were heated at 100°C for 2 min and then tested for their ability to activate calmodulin-depleted phosphodiesterase. As shown in Fig. 5, the heat-treated axoneme fraction stimulated the phosphodiesterase activity to the same extent as bovine brain calmodulin; this activation was dependent upon Ca^{++} and inhibited by 60 μM fluphenazine. Assuming that the calmodulin in the axonemes activated phosphodiesterase with the same efficiency as purified brain calmodulin or *Chlamydomonas* cell body calmodulin, then the isolated axonemes contained ~3 μg of calmodulin/mg protein. Likewise, the membrane plus matrix fraction activated phosphodiesterase to the same extent as brain calmodulin, and this activation was also inhibited by EGTA or 60 μM fluphenazine (results not shown). The membrane plus matrix fraction contained ~6 μg calmodulin/mg protein. Calmodulin appeared to be almost equally distributed between the axoneme and membrane plus matrix fractions; this distribution was not altered when demembranation was carried out in the presence of EGTA instead of EDTA.

Comparison of Calmodulin and Low Molecular Weight Subunits of Dynein ATPases

Jamieson et al. (26) found that calmodulin was present in partially purified preparations of *Tetrahymena* dynein. Dyneins isolated from *Chlamydomonas* axonemes by KCl extraction

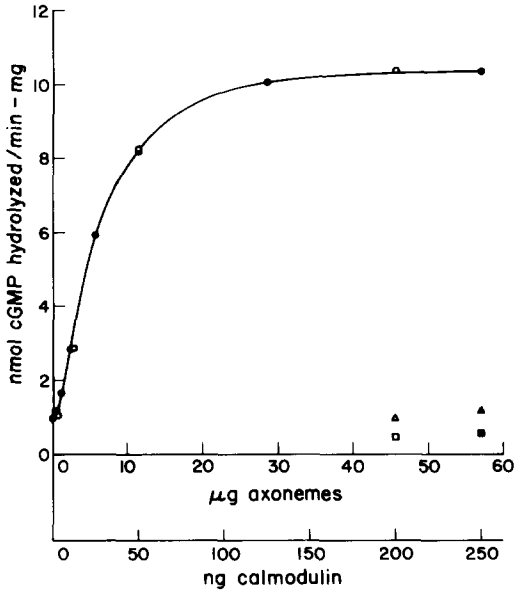

FIGURE 5 Activity of bovine brain cyclic nucleotide phosphodiesterase in the presence of various amounts of isolated axonemes (○) or bovine brain calmodulin (●). Assays were performed simultaneously, and the data for axonemes superimposed on the activation curve for brain calmodulin. In both cases, activation of phosphodiesterase was inhibited by 60 μM fluphenazine (△, axonemes; ▲, brain calmodulin) or 10 mM EGTA (□, axonemes; ■, brain calmodulin). Phosphodiesterase activity is expressed as nanomoles cGMP hydrolyzed per minute-milligram of protein.

followed by sucrose density gradient centrifugation contain several low molecular weight subunits of approximately the same size as calmodulin (33 and footnote 1); to investigate the possibility that one of these subunits might actually be calmodulin, we compared the electrophoretic mobility of these polypeptides with that of purified *Chlamydomonas* calmodulin. As can be seen in Fig. 6, calmodulin coelectrophoresed with one of the low molecular weight subunits of 18S dynein in the presence of Ca^{++}. However, the mobility of this component did not change in the absence of Ca^{++}, indicating that this polypeptide is not identical with calmodulin.

DISCUSSION

In the present study, we found that fluphenazine-Sepharose affinity chromatography of an extract of *Chlamydomonas* cell bodies yielded a single polypeptide which was remarkably similar to mammalian calmodulin in all physical and biochemical properties investigated. These similarities included (*a*) reversible, Ca^{++}-dependent binding to the phenothiazine fluphenazine, (*b*) Ca^{++}-dependent activation of cyclic nucleotide phosphodiesterase, (*c*) heat stability, and (*d*) electrophoretic mobility in SDS-polyacrylamide gels in the presence and absence of Ca^{++}. On the basis of these properties, we consider this protein to be a true calmodulin. This protein is probably the one previously detected in *Chlamydomonas* by means of a radioimmunoassay specific for calmodulin (10).

Although the radioimmunoassay of Chafouleas et al. (10) indicated significant levels of calmodulin in a supernate derived from a boiled sonicate of *Chlamydomonas*, they could detect no phosphodiesterase-stimulating activity in the same preparation. In contrast, we found that purified *Chlamydomonas* calmodulin activated bovine brain phosphodiesterase 10- to 11-fold. The most likely explanation for the difference between our findings and those of Chafouleas et al. (10) is that their crude preparation contained a heat-stable inhibitor of the phosphodiesterase. Recently, Sharma et al. (36) described a heat-stable protein from bovine brain that inhibited cyclic nucleotide phosphodiesterase by binding to calmodulin; a similar protein may occur in *Chlamydomonas*. Such inhibitors are likely to be calmodulin-activated enzymes or proteins whose functions have yet to be defined.

Burgess et al. (9) reported that bovine brain calmodulin migrated in SDS-polyacrylamide gels with an apparent molecular weight of 21,000 when the sample buffer, reservoir buffer, and gel all contained EGTA, and with an apparent molecular weight of 15,000 when the EGTA was replaced with Ca^{++}. We found that a Ca^{++}-induced shift in the electrophoretic mobility of calmodulin occured even if Ca^{++} or EGTA were included in only the sample buffer. The shift under these conditions was smaller than that observed by Burgess et al. (9); however, changing the components of only the sample buffer permitted comparison of the mobilities of proteins in the presence and absence of Ca^{++} in the same gel, and greatly facilitated comparisons of bands in crude fractions electrophoresed under the two conditions.

Our identification of calmodulin in the axoneme and membrane plus matrix fractions of the flagellum is based in part on our findings that these fractions contained a protein that comigrated with *Chlamydomonas* calmodulin in SDS-polyacrylamide gels in both the presence and absence of Ca^{++}. To the best of our knowledge, the large Ca^{++}-induced shift in mobility exhibited by this protein is unique to calmodulin. It should be noted that three other Ca^{++}-binding proteins—carp parvalbumen, troponin C, and bovine brain S-100b—undergo no change in electrophoretic mobility in SDS-polyacrylamide gels in the presence and absence of Ca^{++} (9).

Additional evidence for the presence of calmodulin in the flagellar fractions came from our findings that the heat-treated

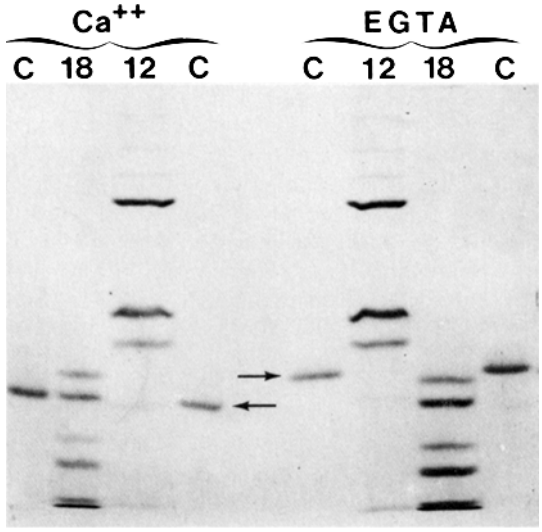

FIGURE 6 Portion of an SDS-polyacrylamide slab gel of purified *Chlamydomonas* calmodulin (*C*) and partially purified 12S (*12*) and 18S (*18*) dynein ATPases electrophoresed in the presence of 1 mM Ca^{++} or 1 mM EGTA, as indicated. Arrows indicate positions of calmodulin under the two conditions. The bands in the 18S dynein fraction all represent low molecular weight subunits of the 18S dynein; the darker bands in the 12S fraction represent components of the 12S dyneins.[1] None of the low molecular weight dynein subunits underwent a Ca^{++}-induced shift in mobility.

fractions activated cyclic nucleotide phosphodiesterase in a Ca^{++}-dependent, fluphenazine-sensitive manner. Such a behavior in the phosphodiesterase assay is a distinguishing feature of calmodulin (46). Both the electrophoretic data and the phosphodiesterase studies, therefore, clearly indicated that calmodulin was present in the axoneme and membrane plus matrix fractions.

We do not yet know the exact location or locations of calmodulin within the flagellum. Interestingly, the fact that calmodulin appeared to be present in axonemes isolated in the presence of EGTA or EDTA indicates that some of the calmodulin remains bound to an axonemal component even in the absence of Ca^{++}. An analogous situation exists in the brush border of chicken intestinal epithelial cells, where calmodulin is tightly associated with the microvillus core in the absence of Ca^{++} (23).

Jamieson et al. (26) found that calmodulin was present in partially purified preparations of dynein from *Tetrahymena*; more recently, these investigators have demonstrated that the dyneins of *Tetrahymena* contain a calmodulin binding site and are activated by calmodulin.[4] *Chlamydomonas* dyneins contain several low molecular weight subunits (33 and footnote 1); however, none of these comigrated with *Chlamydomonas* calmodulin in both the presence and absence of Ca^{++}. Calmodulin is, therefore, not associated with *Chlamydomonas* dynein as isolated by our procedures. This does not rule out the possibility that calmodulin may associate reversibly with the dyneins of this organism *in situ*. Alternatively, dynein *in situ* may be associated with a protein closely related to calmodulin which shares protein binding domains and functional properties with calmodulin. If stripped of that subunit in vitro, dynein might then bind and be activated by exogenous calmodulin. The subunit of the 18S dynein that coelectrophoresed with calmodulin only in the presence of Ca^{++} might be such a protein. As noted above, other members of the troponin C superfamily of Ca^{++} binding proteins do not exhibit the Ca^{++}-induced shift in mobility in SDS-polyacrylamide gels (9).

The occurence of calmodulin in the *Chlamydomonas* flagellum suggests that it is involved in the regulation of one or more of the Ca^{++}-dependent phenomena that occur in this organelle. Further evidence for a possible role for calmodulin in these processes comes from a recent study of Hirschberg and Hutchinson (22) on the effect of chlorpromazine on the phototaxis and motility of *Chlamydomonas*. These investigators found that, at moderate light intensities, 6 μM chlorpromazine caused a reversal in the direction of phototaxis and 50 μM chlorpromazine completely inhibited motility. The latter effect occurred in that concentration range at which chlorpromazine binds to calmodulin and inhibits the activation of bovine brain phosphodiesterase (30, 43) and adenylate cyclase (8); inhibition of motility in *Chlamydomonas* may, therefore, have been a result of binding of the drug to calmodulin with a consequent effect on one of the enzyme systems involved in flagellar movement. Significantly, Reed and Satir[5] have recently found that 25 μM trifluoperazine reversed the Ca^{++}-induced arrest of reactivated cilia of detergent permeabilized mussel gill lateral cells; this result strongly suggests that calmodulin is involved in the regulation of ciliary activity in these cells. Studies are currently under way to determine if calmodulin has any direct role in either the motility or the Ca^{++}-induced change in waveform of isolated, reactivated axonemes of *Chlamydomonas* (3).

Chlamydomonas is one of the most primitive organisms from which calmodulin has been isolated. The fact that *Chlamydomonas* calmodulin is similar to or identical with mammalian calmodulin in all biochemical and biological properties tested, which include immunological cross-reactivity (10), phenothiazine binding, and activation of cyclic nucleotide phosphodiesterase, indicates that the structure of the protein has been very highly conserved throughout evolution. This is in good agreement with studies on the primary structures of calmodulin from two vertebrates (15, 21, 42) and a coelenterate (39), and would be expected for a protein involved in multiple diverse functions of fundamental importance. Calmodulin is thought to be the most ancient of a number of related Ca^{++}-binding proteins (13); it will, therefore, be particularly interesting to obtain information on the amino acid sequence of calmodulin from *Chlamydomonas*.

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